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Packed-microbore supercritical fluid chromatography with flame ionization detection of abused vegetable oils

JOHN E. FRANCE, JANET M. SNYDER and JERRY W. KING*

Food Physical Chemistry Research, Northern Regional Research Center, Agricultural Research Service, United States Department of Agriculture, 1815 North University Street, Peoria, IL 61604 (U.S.A.)

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ABSTRACT

Packed-column supercritical fluid chromatography with flame ionization detection (SFC–FID) has been applied to the analysis of abused vegetable oils. Previous studies have shown that packed-column SFC–FID of polar solutes is difficult due to the unshielded surface silanols on SFC stationary phases. The use of a polymeric resin-based stationary phase or high-temperature water saturation with a bonded-silica column eliminates the problem. With the use of the polymeric resin-packed column, silanophilic interactions are non-existent. High-temperature water saturation of the carbon dioxide mobile phase provides water as a modifier at levels of 2 to 3 mole%, levels previously unattained in packed-column SFC. At this concentration, water notably minimizes fatty acid interactions with the bonded-silica column, yet allows the use of FID. These two chromatographic approaches are illustrated on lipid-containing samples such as oil from storage-abused soybeans and a commercial frying fat. Chromatograms obtained by packed-column SFC–FID are compared with separations attained using capillary-column SFC. Packed-microbore SFC–FID results show a linear correlation with free fatty acid content determined by a standard titration method.

INTRODUCTION

As a complementary technique to high-performance liquid chromatography (HPLC) and gas chromatography (GC), supercritical fluid chromatography (SFC) is making significant contributions in specific application areas, such as in the separation of relatively non-polar polymers [1,2] and of high-boiling petrochemical mixtures [3,4]. However, SFC has yet to establish itself in other areas such as bioanalysis [5]. A major advantage of SFC is that it facilitates the use of flame ionization detection (FID), thereby allowing universal and sensitive detection of organic eluents. Since most lipids lack chromophores, the above feature makes SFC–FID quite appealing for lipid analysis.

The majority of SFC–FID applications for separating and quantitating acyl-containing lipid classes have utilized capillary columns [6–11]. When moderate separation efficiencies are acceptable, packed-column SFC would be preferred for repetitive analyses of minor components. There are two major reasons for such a preference. Packed-column SFC can accommodate higher sample loadings (no split

is required). It also has an advantage over open tubular capillary SFC with regard to the number of plates generated per unit time [12]. However, most packed-column SFC analyses have required the use of organic modifiers to achieve good chromatographic separations of polar solutes, such as free fatty acids. The use of organic modifiers, however, precludes FID.

In this study, we have evaluated two methods that overcome this problem. One employs a column with a polymeric resin stationary phase (Omni-Pac μPRN-300). The other method incorporates water, an FID-compatible modifier, into the supercritical carbon dioxide mobile phase with the use of a polymerically cross-linked octylsilyl silica column (Deltabond Octyl). Water-saturated carbon dioxide has been reported to reduce peak tailing of polar solutes on bonded-silica columns [13]. Chromatograms obtained on these microbore columns are compared to those obtained on an open tubular capillary column having an octyl cross-linked stationary phase (SB-octyl).

Many components in commercial fats and oils are of analytical interest. Free fatty acid content is one. In crude oils, it is used as a guide for processors to optimize alkali refining [14] or to evaluate extent of seed damage [15]. In frying fats, the free fatty acid content is one parameter that may be used as a guide to the replacement of oil in fryers [16]. The standard method for the determination of free fatty acids in oils is a titration procedure [17]. In this study we show that data from packed-column SFC–FID can be correlated with titration values. SFC–FID provides information in addition to fatty acid content in the oil. SFC–FID can also provide information on diglyceride content as well as detect the presence of other organic components in the oil.

EXPERIMENTAL[a]

Samples

Crude soybean oils were obtained from soybean seeds that were subjected to varying degrees of abuse [15]. The test oil samples were characterized for free fatty acid content by a standard titration method [17]. Fresh and used frying fats were obtained from a local restaurant.

Instrumentation

Packed-column SFC–FID was accomplished on two columns, a Deltabond Octyl microbore column, 150 mm × 1 mm I.D. (Keystone Scientific, State College, PA, U.S.A.) and an Omni-Pac μPRN-300 microbore column, 150 mm × 0.75 mm I.D. (Dionex, Sunnyvale, CA, U.S.A.). The supercritical fluid chromatograph consisted of a syringe pump (SFC-500 Microflow, Isco, Lincoln, NE, U.S.A.) with the cooling jacket refrigerated by a recirculating bath (RTE-110, Neslab, Portsmouth, NH, U.S.A.). The pump was controlled by a personal computer (IBM PC-XT) via an interface with associated software (Chemresearch SFC-500 pump controller, Isco). Carbon dioxide (SFC grade, Scott Specialty Gases, Plumsteadville, PA, U.S.A.) was supplied in cylinders with syphon tubes. Samples were injected using a valve (CI4W,

[a] The mention of firm names or trade products does not imply that they are endorsed or recommended by the United States Department of Agriculture over other firms or similar products not mentioned.

Valco, Houston, TX, U.S.A.) equipped with a rotor having a 0.5 μl internal injection volume. A gas chromatograph (Hewlett-Packard 5700A, Palo Alto, CA, U.S.A.) equipped with a conventional FID was used in these studies. Chromatograms were recorded with an integrator (Model 4290, Spectra Physics, San Jose, CA, U.S.A.). Fatty acid peaks were identified by co-injecting standards with the samples. Diglyceride and triglyceride retention times were identified by standards.

The water-saturation precolumn was constructed of 14 cm × 7 mm I.D. HPLC column tubing attached to fritted reducing unions (SS-600-6-1ZV, Swagelock, Solon, OH, U.S.A.). It was placed just before the injection valve in the carbon dioxide feed line from the pump. The packing material, a neutral alumina, (80–200 mesh, Fisher Scientific, Fairlawn, NJ, U.S.A.) was packed dry. Deionized water was added to the sorbent at proportions under 10 wt% of the alumina by removing one union and pipetting the water directly onto the alumina. Depletion of the water in the precolumn was avoided by using equilibrium solubilities [18] to estimate when the addition of water to the precolumn was required. The water was equilibrated with the sorbent by sealing the column and holding it at 60°C overnight. An HPLC column heater (Bio-Rad, Richmond, CA, U.S.A.) was used to heat the water-saturation precolumn. The water-saturation precolumn was used only with the Deltabond C_8 column.

Capillary SFC–FID was performed on a 15 m × 50 μm I.D. SB-Octyl-50 column with a 0.25-μm film thickness (Dionex, Lee Scientific, Salt Lake City, UT, U.S.A.) using a Lee Scientific Model 501 supercritical fluid chromatograph controlled by an IBM PC-AT computer. The crude oils or frying fats were diluted to 5% (v/v) in hexane prior to injection. Samples were injected using an actuated valve with an internal loop of 0.2 μl. A dynamic split was used at a ratio of 10:1. A frit restrictor was used to maintain column backpressure. FID was accomplished with the detector operating at 350°C. Chromatograms were recorded on an integrator (HP3396A, Hewlett-Packard, Avondale, PA, U.S.A.).

RESULTS AND DISCUSSION

Comparison of columns

Fig. 1 shows a comparison of chromatograms for fresh and used frying fat samples obtained with the C_8 bonded-silica column operated with a room temperature water-saturation precolumn (a,b), the polymeric resin column (c,d) and the C_8 capillary column (e,f). Operating conditions for the columns were selected so as to limit analysis time to 35 min or less. On each column, low levels of fatty acids (0.2%) were observed in the used frying fat. The fresh frying fat samples showed no chromatographic peaks due to fatty acids at this sample dilution. Some differences in column behavior are apparent in the chromatographic profiles. The packed C_8 bonded-silica column showed severe tailing of the fatty acids. Tailing of the fatty acid peaks was not problematic on the polymeric resin column or the C_8 capillary column. Diglycerides were eluted in a band separate from the triglycerides on the C_8 packed and capillary columns, but not on the polymeric resin column.

Chromatograms obtained on crude soybean oil samples using the three column types are compared in Fig. 2. Again, it is observed that the C_8 packed and capillary columns were able to separate the diglycerides from the triglycerides. Such a separation was not possible on the polymeric resin column, even though various chromato-

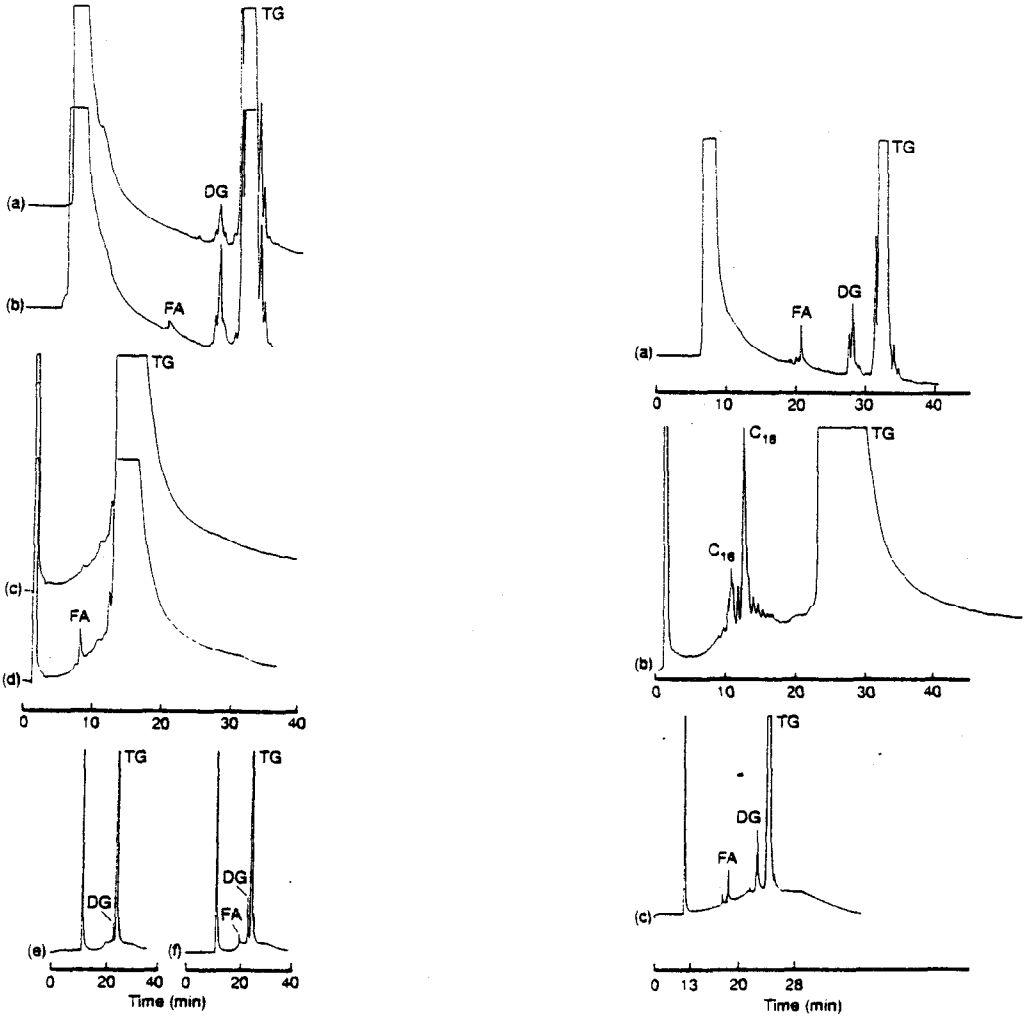

Fig. 1. SFC–FID chromatograms of commercial frying fat: 47 mg/ml in isooctane; 400°C detector. (a) Fresh frying fat; (b) used frying fat. Deltabond Octyl, 100°C column oven, linear pressure ramp: 9.0 MPa, hold for 5 min, 9.0 to 35.4 MPa over 30 min, hold at 35.4 MPa, water precolumn at 25°C. (c) Fresh frying fat; (d) used frying fat. Omni-Pac μPRN-300, 170°C column oven, linear pressure ramp: hold at 19.9 MPa for 3 min, 19.9 to 41.6 MPa over 30 min, hold at 41.6 MPa. (e) Fresh frying fat; (f) used frying fat. Capillary SB Octyl, 120°C column oven, linear density ramp: hold at 0.28 g/ml for 11 min, 0.28 to 0.40 g/ml over 6 min, 0.40 to 0.70 g/ml over 3 min, hold at 0.70 g/ml for 10 min. FA = Fatty acids, DG = diglycerides, TG = triglycerides.

Fig. 2. SFC–FID chromatograms of oil from storage-damaged soybeans, 16% moisture for 27 days; 400°C detector, 0.5 μl injection, 100°C column oven, injection solution is oil–hexane (5:95, v/v). (a) Deltabond Octyl, linear pressure ramp same as Fig. 1a, water precolumn at 25°C; (b) Omni-Pac μPRN-300, linear pressure ramp: 15.2 to 38.2 MPa over 30 min, hold at 38.2 MPa; (c) capillary SB Octyl, (same conditions as in Fig. 1e). C_{16} = Fatty acid of carbon chain length 16. C_{18} = fatty acids of carbon chain length 18, other peak labels same as Fig. 1.

graphic conditions were attempted. The polymeric resin column does show a set of small unidentified peaks superimposed on the fatty acid peaks (the two larger peaks). These compounds are presumed to be the same ones that elute just ahead of the fatty acids on the packed C_8 column.

Larger sample loadings could not be tolerated with the capillary column because of split inconsistencies with more concentrated samples. Generally, split injections are necessary for capillary SFC because of sample-loading restrictions [19].

Comparison of soybean oil samples

Neat soybean oil injected onto the polymeric resin column allows for direct analysis of the oils for trace components as well as compounds generally masked by the solvent peak. The SFC chromatograms of two neat oil samples from soybeans subjected to different levels of storage abuse are compared in Fig. 3. Fatty acid levels in these two samples showed the expected trend. The oil from soybeans that were storage abused to a greater degree (Fig. 3a) has a higher fatty acid level than the oil from beans subjected to less abuse (Fig. 3b). One can also observe the presence of two unknown peaks eluting prior to the C_{16} and C_{18} fatty acid peaks in Fig. 3a. Resolution of the various unsaturated acids from each other or from the saturated acids could not be accomplished with any of the columns.

With the set of oils obtained from storage-abused soybeans, a calibration curve could be constructed of free fatty acid contents (%FFA) as measured by titration *versus* the ratio of FFA peak areas to the total peak area. Linear regression yielded the equation, $y = 0.0068x + 0.0007$ with $R^2 = 0.94$. Titration acid numbers of the abused oils ranged from 0.9 to 2.34% as displayed in Table I. SFC–FID peak areas were obtained using the C_8 bonded-silica column with an 85°C water precolumn. Other SFC conditions are as reported in Fig. 1a.

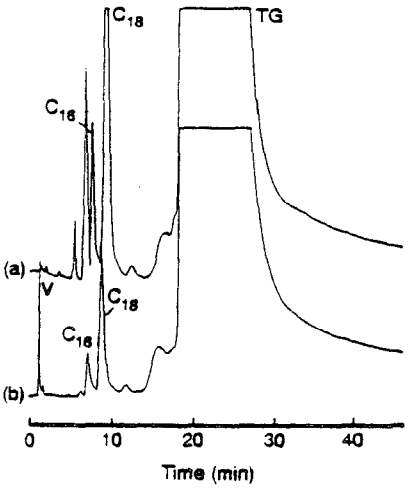

Fig. 3. SFC–FID chromatograms of neat oil injections; 0.5 μl injection, 100°C column oven, 400°C detector, linear pressure ramp: hold at 19.9 MPa for 1 min, 19.9 to 40.2 MPa over 29 min, hold at 40.2 MPa. (a) Oil from storage-damaged soybeans, 20% moisture for 28 days; (b) oil from storage-damaged soybeans, 16% moisture for 9 days. V = volatile compound, other peak labels same as Fig. 2.

TABLE I

COMPARISON OF SFC–FID WITH TITRATION DETERMINATIONS

FFA Peak area[a] / Total peak area	Titration[b] (% FFA)
0.0065	0.90
0.0081	1.04
0.0150	2.02
0.0162	2.34

[a] Ratio of free fatty acid peak areas to the total integrated peak area excluding the solvent peak.
[b] Percentage free fatty acid content as determined by titration and reported as weight percent oleic acid.

Addition of water modifier

Previous studies [13,20,21] have shown the advantages of using water as a modifier for silica-based packed columns. Similar benefits were also observed in this study as shown in Fig. 4. With dry supercritical carbon dioxide, the two fatty acid peaks are unresolved and show severe tailing at a retention time of approximately 22 min (Fig. 4a). Note that the diglyceride peaks are also unresolved and appear as a single peak. Addition of water from the precolumn at room temperature significantly improved the separations of the fatty acids and the diglycerides (Fig. 4b). Optimal chromatography was obtained with the water precolumn heated at 100°C, the highest

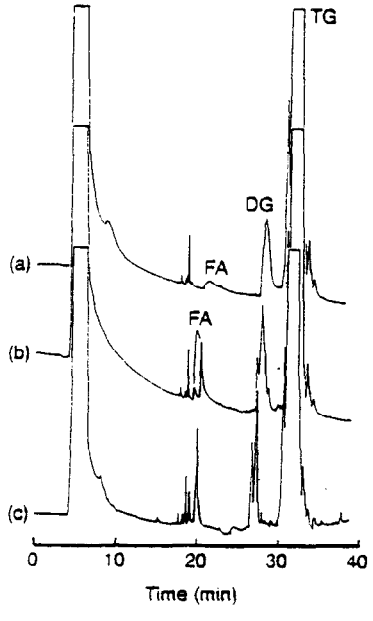

Fig. 4. SFC–FID chromatograms of oil from storage-damaged soybeans (20% moisture for 28 days) with various amounts of water modifier: 0.5 μl injection, 100°C column oven, 400°C detector, linear pressure ramp: hold at 9.0 MPa for 5 min, 9.0 to 35.4 MPa over 30 min, hold at 35.4 MPa. (a) No water precolumn; (b) water precolumn at 25°C; (c) water precolumn at 100°C. Peak labels same as Fig. 1.

precolumn temperature tested (Fig. 4c). To our knowledge, this is the first reported use of a water-saturator precolumn at elevated temperatures for packed-column SFC. Note that minimal tailing of the fatty acids is observed. The peaks eluting just prior to the fatty acids are not affected by the modifier indicating that they contain no functional groups that strongly interact with the chromatographic surface. Co-injection of fatty acid methyl esters with the samples showed that these compounds are not methyl esters of the fatty acids. It is speculated that the compounds may be the corresponding aldehydes of the C_{16} and C_{18} fatty acids. Capillary headspace GC has confirmed the presence of shorter-chain aldehydes in these oils [15]. The unidentified compounds may play a role in oil flavor or stability. Further analysis by other detector schemes such as mass spectrometry or Fourier transform infrared spectrometry will be required to ascertain the identity and importance of these oil components.

The quantity of water that is soluble in carbon dioxide increases exponentially with temperature [18]. Experimental data also indicate that pressure has a limited effect on water solubility at a given temperature when the pressure is above 15 MPa. The water precolumn temperatures of 25 and 100°C utilized for the chromatograms in Fig. 4 produced mole fractions of water in the supercritical carbon dioxide of approximately 0.3–0.4 mole% and 2.3–3.0 mol%, respectively. The dramatic improvement in peak shape of the fatty acids or other polar analytes has been attributed to the filling of the pores of the stationary phase with water [21] as well as to the deactivation of active sites by the water modifier [22,23].

The above results illustrate several of the advantages that attend the use of SFC–FID over conventional chromatographic methods of lipid analysis [24,25]. First, the variable elution power of supercritical carbon dioxide permits simultaneous analysis of the three major lipid classes (fatty acids, diglycerides, triglycerides) without having to resort to multiple analysis methods. Second, derivatization of the fatty acids is not required. Third, injections of neat oils can be accommodated by packed-column SFC, thereby allowing early-eluting trace components to be detected in samples that are predominantly composed of triglycerides.

CONCLUSIONS

Packed-column SFC–FID has been shown to be a potentially valuable technique in the analysis of abused vegetable oils. A new polymeric microbore packed column has been evaluated for the chromatographic analysis of lipid samples and has been shown to be compatible with the injection of neat oil samples. The addition of water at high presaturation temperatures permits the analysis of oil samples containing free fatty acids by reducing the fatty acid interactions with the residual surface silanol groups of a bonded-silica column. Packed-column SFC–FID results can be correlated with the free fatty acid content of an oil as determined by titrimetric methods.

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